

ENHANCEMENT OF FERTILITY AND LIBIDO IN MALE SPRAGUE DAWLEY RATS FOLLOWING THE ADMINISTRATION OF AQUEOUS EXTRACT OF *Lunasia amara*

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ABSTRACT

Lunasia amara (sanrego) is a folk medicinal herb which has been consumed to treat male fertility and libido. This study aims to investigate the effect of *L. amara* aqueous extract on libido, sperm quality, serum testosterone level, antioxidant enzyme activity and histology of testis. Two groups (n=6) were given distilled water (control) and aqueous extract of *L. amara* at a dose of 60 mg/kg/body weight respectively daily for 42 days. On day 43, rats were subjected to sexual behavioural test before sacrificed for sperm quality and testosterone analysis. Cauda epididymis was isolated for sperm quality analysis whereas serum was collected to determine the level of testosterone. Histology of testes and antioxidant enzyme activities were also investigated. Results showed the administration of *L. amara* aqueous extract significantly ($p < 0.05$) increased the mount frequency compared to normal rats. Sperm count, motility, viability, testosterone level and testicular antioxidant enzyme activities in treated rats showed significantly ($p < 0.05$) increased compared to control. Section of seminiferous tubules in treated group showed the increasing of spermatozoa density compared to the normal group. In conclusion, *L. amara* aqueous extract is effective in improving libido, sperm quality, serum testosterone level and testicular antioxidant enzyme activities while increased the density of spermatozoa.

Key words: *Lunasia amara*, libido, sperm quality, sexual behaviour, testosterone, antioxidant enzyme activity

INTRODUCTION

Approximately 50% of infertility problems are due to men and 40-60% of reduced male fertility cases are caused by the abnormality of sperm parameters such as decreased in number of sperm (oligozoospermia), decreased of sperm motility (asthenozoospermia) and morphology (teratozoospermia) (Dohle *et al.*, 2005). World Health Organisation (2000) defined infertility as inability of a sexually active, non-contracepting couple to achieve pregnancy in one year.

Aphrodisiac agent generally regarded as a substance that increase libido or enhances sexual performance and it could be act physiologically, enhancing erection through hormonal changes, increased blood flow and smooth muscle-relaxing properties (Sandroni 2001; Singh *et al.*, 2013). Nowadays, aphrodisiac agent can be either natural or synthetic product. Natural product such ginseng, maca and tongkat Ali are well known herbs to improve male libido (Lewis and Elvin-Lewis, 2003). Sildenafil and tadalafil are common synthetic

product of aphrodisiac that can give side effects such as headache, muscle pain and blurred vision to patient (Sandroni 2001). A study of herbs that potentially increased libido and fertility should be done as an alternative approached.

Sanrego or scientific name *Lunasia amara* belongs to the family Rutaceae (Darise, 1999). It has been claimed to have aphrodisiac properties to treat male sexual desire and also effective in enhancing male fertility. There are many studies conducted regarding to the effect of *L. amara* on male reproductive system. Previous research by Muhtadi (1999) showed that *L. amara* has aphrodisiac property which was effectively enhancing sexual desire. Nurbaeti (2000) reported that alkaloid fraction of *L. amara*, given orally to the male chicks for 15 days has androgenic activity by increasing testicular weight.

Previously the effects of *L. amara* aqueous extract at doses 30, 60 and 90 mg/kg were studied on male rat. The treatment at dose 60 mg/kg/day were the most effective dose for *L. amara* to increase male rat libido and fertility significantly ($p < 0.05$) (Muhamad-Ja'far and Mahanem, 2009). However,

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the relation between testosterone level, antioxidant enzyme activity with libido and sperm quality in male rat treated with *L. amara* aqueous extract has not been reported yet. Therefore, the present study was designed to investigate the mechanism of *L. amara* in enhancing male libido and fertility.

MATERIALS AND METHODS

All experiments were carried out in accordance with guidelines approved by ethical committee of UKM Animal Ethics which includes minimizing the number of animals used and their suffering.

Extraction of *L. amara* stem

Aqueous extract of *L. amara* stem was prepared according to Gonzales *et al.* (2006). The *L. amara* stem was cut into small pieces, dried and ground into powder. About 200 g of dried *L. amara* was boiled at 80°C in 1000 mL of distilled water for 60 minutes. The extract was left standing to cool and filtered before freeze-dried. The extract was stored at 4°C until used.

Animals

Sprague-Dawley male rats aged eight weeks were obtained from Animal House, Universiti Kebangsaan Malaysia. A total of 12 rats were divided into two groups. Group 1 (n=6) were administered with 1 mL distilled water and served as control and group 2 (n=6) administered with 60 mg/kg body weight of *L. amara* aqueous extracts. All treatments were carried on orally using force feeding needle, once daily for 42 consecutive days.

Sexual behavioural test

On day 43, the rats were subjected to sexual behavioural test according to Agmo (1997). Female rats were brought into oestrus phase for sexual activity through subcutaneous injections of 20 µg/rat of estradiol benzoate and 1 mg/rat of progesterone, 52 hours and 4 hours respectively prior to pairing. Male rat was introduced to female rat and the number of mount frequency was counted within 10 minutes.

Sperm sample preparation

The rats were sacrificed and the cauda epididymis was isolated for sperm analysis. The cauda epididymis were minced using anatomical scissors, suspended in 15 mL of Biggers-Whitten-Whittingham (BWW) medium (Biggers *et al.*, 1971) prior to incubation in 5% CO₂ incubator for 30 min at 37°C to allow sperm to swim up. Sperm count was assessed using 'Improved Neubauer Haemocytometer' based on WHO (2010) with modification.

Sperm viability and motility was determined as per WHO laboratory manual (2010).

Testosterone assay

Blood samples from all rats were collected from cardiac puncture. Serums were separated from blood samples by centrifugation at 2500 rpm for 10 minutes and it was stored at -20°C until used. The testosterone levels were determined using Testosterone Enzyme Immunoassay Kit (EIA) from Cayman Chemical Company, USA.

Antioxidant enzyme activity assay

Homogenization of testis tissue was conducted according to Choi *et al.* (2008). Homogenized testis (200 mg) was mixed with 1 mL phosphate buffer saline (pH 7.4). The sample was centrifuged at 10,000 × g for 15 minutes at 4°C and the supernatant was removed for antioxidant enzyme assay. The antioxidant enzyme activity of Superoxidase Dismutase (SOD) and Catalase (CAT) were determined from the supernatant using SOD and CAT Assay Kit from Cayman Chemical Company, USA. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of superoxide radical (U/mL). One unit of CAT is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C.

Histology of testes

The testes were fixed in a Bouin's solution for overnight, washed in 0.9% NaCl, dehydrated through graded concentration of ethanol series, cleared in toluene and embedded in paraffin wax. Tissues were sectioned at 5 µm thicknesses and stained with Hematoxylin and Eosin (H&E) stains. The slides of testicular spermatogenesis were observed and evaluated qualitatively under light microscope.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 18 was used for statistical analysis. The data are presented as mean ± standard error of means (SEM) and statistical significance was tested by One-Sample t test. $P < 0.05$ was considered a statistically significant difference between the *L. amara* treated group and the control group.

RESULTS

Sexual behaviour assessment

Sexual behaviour from treated and control group were shown in Fig. 1. The average of the frequency of male rat mounts on female was 2 ± 0.26 for control group and 5.5 ± 0.50 for treated group.

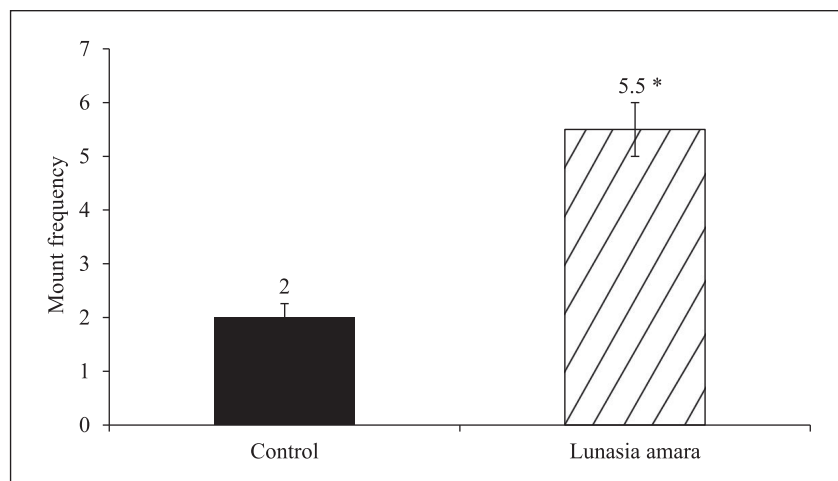


Fig. 1. Effects of *L. amara* aqueous extract on the mount frequency in male rats within ten minutes observation compared to control (*significant difference $p < 0.05$).

Treatment group (60 mg/kg) showed high mounting frequency compared to control group suggesting that *L. amara* aqueous extract has an aphrodisiac property.

Sperm quality assessment

The effect of *L. amara* aqueous extract on male fertility was demonstrated by sperm quality analysis. Fig. 2 and 3 show the effects of *L. amara* aqueous extract on sperm count, viability and motility. The sperm count and viability of *L. amara* aqueous extract group showed significantly ($p < 0.05$) increased at $39.88 \pm 2.33 \times 10^6$ and $82.46 \pm 1.91\%$ respectively compared to control group (Fig. 2).

The percentage of sperm motility showed treated group has significant ($p < 0.05$) increased in progressive (PR) sperm motility at $78.78 \pm 0.72\%$ compared to the control group $67.61 \pm 3.94\%$. Administration of *L. amara* has succeeded in increasing the percentage of progressive sperm motility (Fig. 3).

Testosterone assay

Serum testosterone level was shown in Fig. 4. *L. amara* treatment after 42 days indicated that testosterone level increased compared to control group. Serum testosterone level in treated group (1.10 ± 0.03 ng/mL) was significantly ($p < 0.05$) higher than the control group (0.75 ± 0.12 ng/mL).

Antioxidant Enzyme Activity Assay

Activity of testicular antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were shown in Fig. 5. The level of SOD activity in *L. amara* group resulted a significant ($p < 0.005$) increased (181.98 ± 11.66 U/mL) by 13.7% compared to the control (157.05 ± 15.85 U/mL).

Administration of *L. amara* also significantly ($p < 0.05$) increased the CAT activity (347.75 ± 47.41 U/mL) by 24.93% compared to control (261.05 ± 58.06 U/mL).

Effects of *L. amara* aqueous extract on spermatogenesis

Seminiferous tubular structure in Fig. 6 suggested that administration of 60 mg/kg/day *L. amara* aqueous extract increased germinal cell layer thickness when compared to the control group. The treated group exhibited high density of spermatogenic cells in the lumen (Fig. 6b).

DISCUSSION

The results of this study demonstrated that the administration of *L. amara* aqueous extract with dose 60 mg/kg/day on male rats was significantly ($p < 0.05$) increased the male libido and sperm quality including sperm count, motility and viability compared to control. The result showed that *L. amara* extract has a potential as an aphrodisiac agent for increase the sexual desire or libido in male rats. According to Sandroni (2001), aphrodisiac can have psychological effects when consumed, thereby increasing sexual desire and pleasure through hallucinogenic properties or other mood stimulating properties. We predict that *L. amara* acts psychologically, enhancing erection through hormonal changes, increased blood flow and smooth muscle-relaxing properties.

Pharmacognosy and phytochemistry study revealed that *L. amara* contains steroids. Administration of steroid plant such as *L. amara* stimulates the reproductive organs related to sexual behaviour. Steroid will goes through the process of

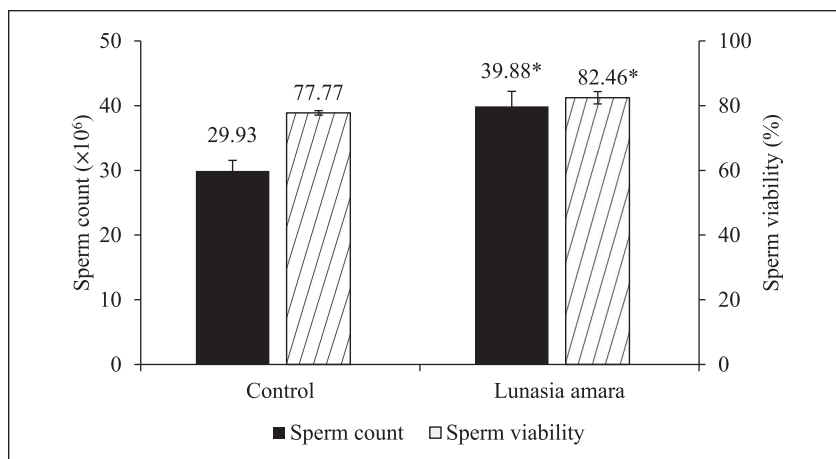


Fig. 2. Effects of *L. amara* aqueous extract on epididymal sperm count and sperm viability compare to control (*significant difference $p < 0.05$).

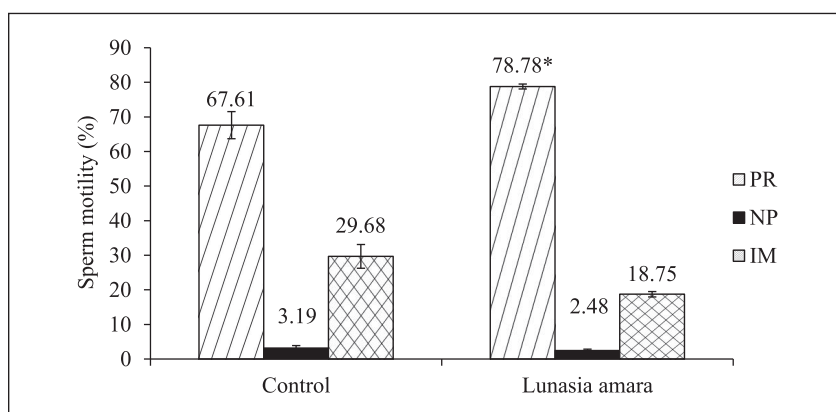


Fig. 3. Effects of *L. amara* aqueous extract on epididymal sperm motility compared to control (*significant difference $p < 0.05$).

Note for the grade of sperm motility according to WHO 2010 :
 PR – Progressive motility (spermatozoa moving actively, either linearly or in a large circle, regardless of speed)
 NP – Non progressive motility (all other patterns of motility with an absence of progression)
 IM – Immotility (no movement)

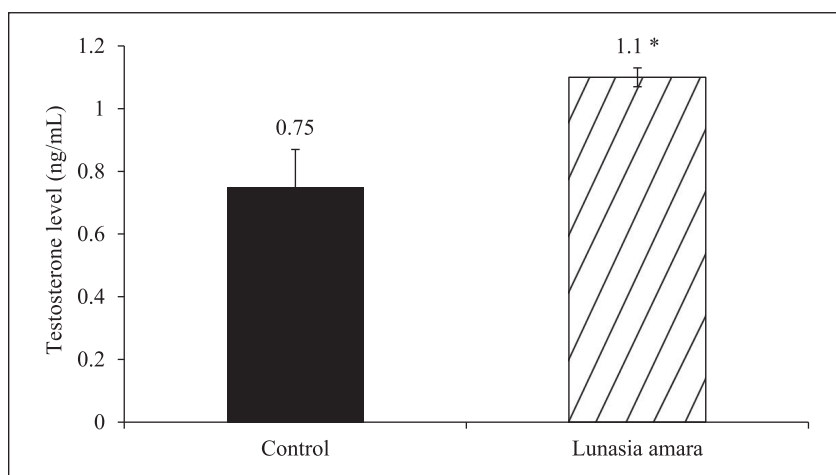


Fig. 4. Effects of *L. amara* aqueous extract on serum testosterone level compared to control (*significant difference $p < 0.05$).

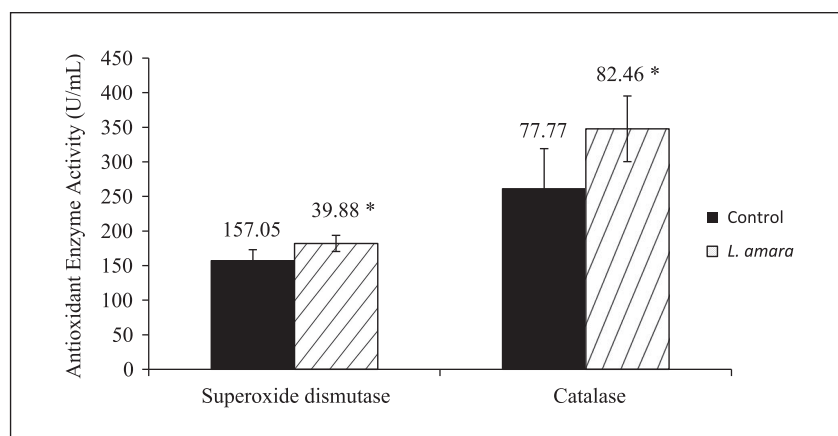


Fig. 5. Effects of *L. amara* aqueous extract on superoxide dismutase and catalase activities compared to control (*shows significant difference, $p < 0.05$).

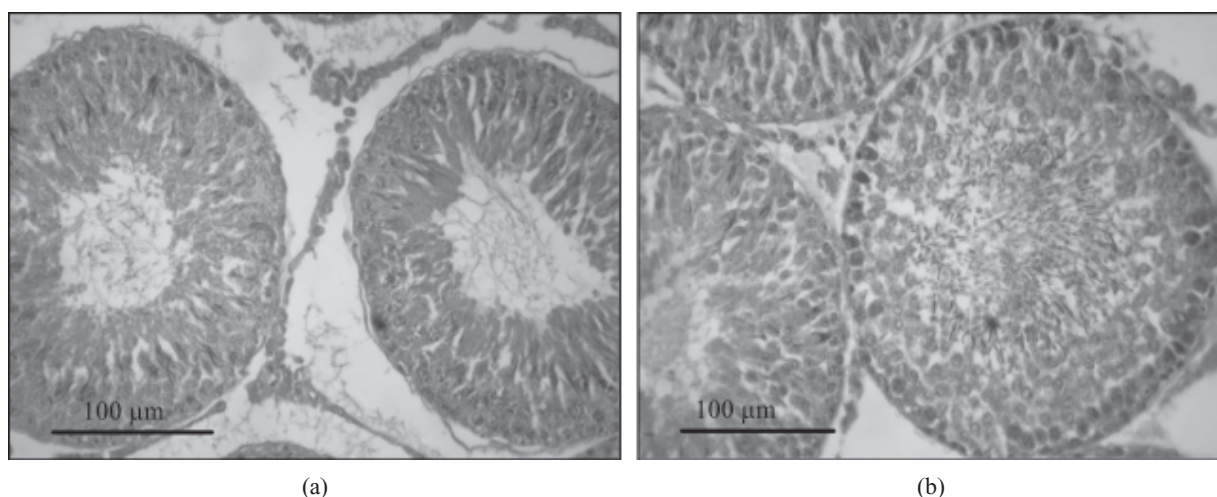


Fig. 6. Section of seminiferous tubules of rats from a) control group and b) treated group with 60 mg/kg/day *L. amara* aqueous extract. Hematoxylin and eosin (H&E) stains (x400).

digestion and absorbed into the bloodstream (Nurlaila, 2000). The presence of steroids in *L. amara* may support the result of male libido, indeed further study on this relation is needed.

Along with the elevation in sperm quality following the oral administration of 60 mg/kg *L. amara* for 42 days, the increase of serum testosterone level was also detected. The highest level of testosterone in *L. amara* group compared to control showed that testosterone has correlate with the increasing of libido and sperm quality. The receptive female is believed to affect the activation of the hypothalamic-pituitary-testicular complex (HPTC) in the male which is indicated by increased serum testosterone and luteinizing hormone levels (Bartke and Dalterio, 1975).

One of the folk medicine in India, *Mucuna pruriens* Linn has similar effect with *L. amara* plant for treating male sexual disorders. *M. pruriens* plant

showed relatively good result in terms of sexual behaviour, libido potency and spermatogenic potential (Suresh *et al.*, 2009). According to McGinnis *et al.* (1989), the correlation of testosterone and aphrodisiac activity can be assess through dopaminergic pathway with the presence of high level of L-Dopa in *Mucuna pruriens*. L-Dopa is a natural amino acid derived from food sources and a precursor for the needed and beneficial neurotransmitter dopamine. Therefore it is important to determine if *L. amara* has a similar mode of action as *Mucuna pruriens*.

The excessive of reactive oxygen species (ROS) generated by cell metabolism may suppressed the ability of sperm function leading to infertility. ROS is physiologically generated during mitochondrial respiration in normal cell metabolism (de-Lamirande *et al.*, 1997). The level of ROS reflects to the highly specific lipid composition of sperm membrane cells

as the main substrate for lipid peroxidation. At the low level of lipid peroxidation, where the ROS is low, the motility and functional ability of sperm cells to interact with zona pellucida will increased. However, the pathological lipid peroxidation of sperm membrane due to the high level of ROS will undergo unbalance oxidative stress in the testes (Aitken *et al.*, 1989; Aitken and Roman, 2008). The presence of the intra and extracellular antioxidants of enzymatic and non-enzymatic system however will scavenge free radicals as self-protection mechanism (Alvarez *et al.*, 1987). Intake of superoxide dismutase supplement was leading to the progressive sperm motility and improved the acrosome reaction (Griveau and Le Lannou, 1997). In this study, the administration of *L. amara* has succeeded in increasing the antioxidant enzyme activity of superoxide dismutase (SOD) and catalase (CAT) suggesting the *L. amara* has anti-oxidative effect that contribute to male fertility.

CONCLUSION

In conclusion, our *in vivo* studies indicate that the aqueous extract of *L. amara* possesses a significant aphrodisiac and fertility effect in male rats. Taken together these results lead us to conclude that the aqueous extract of *L. amara* at dose 60 mg/kg/day significantly increase sperm quality and male libido by elevating antioxidant enzymes activity and improving testosterone level. However, we cannot exclude the possibility that other mechanisms may be responsible for promoting the sperm quality and libido. Further pharmacological and phytochemical studies are currently in progress to investigate the mode of action of *L. amara*.

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